Pak. J. Bot., 42(5): 3497-3505, 2010.

PHYSIOLOGICAL RESPONSES OF VARIOUS WHEAT GENOTYPES TO SALINITY

M. A. KHAN[*], E. ISLAM[1], M.U. SHIRAZI, S. MUMTAZ, S.M. MUJTABA, M. ALI KHAN, A. SHEREEN, M.Y. ASHRAF[2] AND G.M. KALERI

Plant Physiology Division, Nuclear Institute of Agriculture, Tandojam, Pakistan
[1]Nuclear Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad
[2]Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad

Abstract

Effect of NaCl stress on yield and various physiological parameters (leaf area, osmotic potential, glycine-betaine, total sugars and chlorophyll contents) was studied in 7 wheat genotypes (Lu-26s, Sarsabz, Bhittai, KTDH22, Khirman, B-7012 and Bakhtawar) grown under two salinity levels (NaCl 1.5 and 12 dS/m) in the cemented tanks having river sand. Seeds were allowed to germinate under normal condition and salinity treatments were imposed after one week of germination. Salinity was imposed by irrigating the crop at an interval of two weeks or whenever required with 1/4th Hoagland nutrient solution having respective NaCl concentrations. Salinity reduced the grain yield, leaf area and chlorophyll contents however it resulted in an increase in the osmotic potential, glycine-betaine and total sugar contents. The results clearly indicated that under salt stress, genotypes with higher leaf area, osmotic potential, glycine-betaine, total sugar and chlorophyll contents, had more grain yields as compared to the genotypes with lower values for these attributes. On the basis of yield reduction, four genotypes viz., Lu-26s, Sarsabz, Bhittai and KTDH-22 were found to be salt tolerant whereas genotypes V-7012, Khirman and Bakhtawar were designated as sensitive ones. The tolerant genotypes also maintained higher leaf area, osmotic potential, glycine-betaine, total sugar and chlorophyll contents under saline conditions.

Introduction

Soil salinity is the major obstacle for increasing production in cropping areas throughout the world. Salinity limits the productivity of almost all crops and its impact and severity has been exacerbated by the activities of man. According to Wild (2003), about 15% of the total land area of the world has been degraded by soil erosion and physical/chemical degradation including soil salinization. In Pakistan about 6.3 million hectares of land has become saline (Qureshi, 1993; Ghassemi *et al*., 1995), about half of which lies in the canal command area (Rafiq, 1990) while about 40,000 hectares of agriculture land are becoming saline annually.

Wheat (*Triticum aestivum* L.) is an important cereal crop grown all over the world. Also in Pakistan, it is the major staple food and grown on an area of 8.6 million hectares (Anon., 2009). Use of improved varieties and better agronomic practices during the past four decades has boosted the total wheat production of the country from 6.5 million tons in 1970-71 to 23.4 million tons during 2008-09 (Anon., 2009). However, due to increasing salinity and growing population, there is still a need to increase wheat production in the country. Literature available on salt tolerance in wheat varieties suggests that it is a moderately salt tolerant crop with a threshold level of 6-7 dS m^{-1} (Maas, 1986). Apart from the genotypic variations in salt tolerance, response of a particular wheat genotype under saline conditions also varies during different stages of growth (germination, early seedling, vegetative, grain filling and maturity stage) and under different growth conditions (growth cabinets, green house or natural fields) (Khan, M.A., Ph.D. Thesis, 2009).

[*]Corresponding author's e-mail: atharvienna@yahoo.com

In order to grow in saline environments, plants have adapted a number of morphological, physiological and biochemical processes to mitigate the effects of high concentrations of toxic salts and accordingly vary in their ability to tolerate saline conditions. Physiological traits such as potassium selectivity, exclusion and/or compartmentation of sodium and chloride ions, osmotic adjustment by accumulation of organic solutes (proline, glycine-betaine, total sugars) have all been related to salt tolerance of crop plants (Wyn Jones & Storey 1981). The present study has been carried out to find the relationship between yield and some physiological traits of various wheat genotypes grown under saline conditions in the glasshouse.

Materials and Methods

Seeds of seven wheat genotypes were acquired from the Plant Breeding and Genetics Division, Nuclear Institute of Agriculture (NIA), Tandojam for their assessment on the basis of physiological and biochemical parameters. Healthy seeds were selected and surface sterilized with 3% Sodium hypochlorite solution for 15-20 minutes to prevent fungal infection. Then seeds were washed thoroughly with distilled water and sown in cemented tanks having river sand. Seeds were sown in a randomized manner with three replicates. Crop was irrigated at an interval of two weeks or when ever required with $1/4^{th}$ strength Hoagland nutrient solution salinized by common salt (NaCl) to attain salinity levels of 1.5 dS/m (control) and 12.0 dS/m. Electrical conductivity of the solution in tanks was maintained regularly throughout the season. Various physiological parameters were studied at the time of booting stage and crop was taken up to maturity at which grain yield was recorded.

Leaf area was determined by passing fresh leaf samples through Leaf Area Meter (LI-COR - LI-3100, USA). For osmotic potential (OP), the method described by Khan *et al*., (1992) was used. Fresh leaf samples (flag leaf) were taken and immersed in a glass tube. A swab of cotton containing chloroform was placed in the test tube. The test tubes were then left in freezer for over-night to kill the leaf tissues. After 24 hours these tubes were taken out, acclimatized at room temperature and the cell sap was extracted with the help of a syringe. This cell sap was collected in PCR tubes and osmotic potential (OP) was measured by Osmometer (Osmomat-030, Germany).

Glycine-betaine was estimated by the method described by Grieve & Gratan (1983). Dried ground leaves 0.5 g was extracted by shaking mechanically with the known amount of toluene water mixture for 24 hours at 25^{o}C, and filtered through Whatman filter paper No. 1. One ml of the extract was mixed with 1 ml of 2.0 *N* HCl and was mixed thoroughly. After that, 0.5 ml of the reaction mixture was pipetted out in a glass tube and 0.2 ml of potassium tri-iodide solution was added (7.5 g iodine and 10 g potassium iodide dissolved in 100 ml of 1.0 *N* HCl by continuous shaking for 30 minutes then filtered and stored at 25^{o}C). The contents were shaken and cooled in ice bath for 90 minutes with occasional shaking. Two ml of ice cooled distilled water was mixed and then 20 ml of 1-2 dichloro ethane (cooled at 10^{o}C) was added. Two layers were formed which were mixed by passing a continuous stream of air for 1-2 minutes while tubes were still in the ice bath (4^{o}C). The upper aqueous layer was discarded and optical density of organic layer was measured at 365 nm. The concentrations of glycine-betaine were calculated against the standard curve. The blank samples were also developed as above except no glycine-betaine standard or extract.

The total soluble sugars were determined in fresh leaves according to Riazi *et al*., (1985). Fresh leaf samples were chilled to 0^oC immediately after harvest and then frozen at 4^oC within ten minutes. One gram chopped leaf samples were shaken with 10 ml of 80% ethanol (v/v) overnight. In 0.1 ml ethanol extract, 3 ml of freshly prepared anthrone was added, heated at 97^oC for 10 minutes, cooled in ice bath and read in spectrophotometer (Hitachi 150, Japan) at 625 nm.

The chlorophyll was determined according to the method of Lichtenthaler (1987). Fresh leaves were cut into small pieces and extracted overnight with 80% acetone. The extract was centrifuged at 14000 x g for 5 minutes and the absorbance of the supernatant was measured at 645, 663 nm using Spectrophotometer.

All data were processed by statistical software MSTAT-C. Values reported are means of three replicates. Data were tested at significant levels of $p \leq 0.05$ using Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT). Graphical work was carried out using Sigma Plot software v.10.

Results

Application of different salinity levels posed variable effects on grain yield and various physiological traits of all the genotypes.

Effect of salinity on the grain yield plant^{-1}: Salinity levels significantly ($p \leq 0.05$) reduced the grain yield in all genotypes (Fig. 1). Maximum reduction was observed in the genotype Khirman, followed by V-7012 and Bakhtawar. The genotypes Sarsabz, Bhittai and Lu-26s showed better performance as compared to others, whereas KTDH-22 was found as moderately salt tolerant.

Effect of salinity on leaf area and osmotic potential: Data regarding the variation in leaf area is presented in Table 1. It was observed that imposition of salinity caused a significant decrease in leaf area in comparison to control plants however genotypic variations were observed. Maximum reduction in leaf area was noted in Bakhtawar (42.86%) followed by V-7012 and Khirman. Genotypes Lu-26s, Sarsabz and Bhittai proved to be tolerant at this level of salinity and successfully maintained more than 75% of the leaf area when compared with their respective controls.

Application of salinity caused a marked increase (more negative values) in osmotic potential of all the wheat genotypes as compared to control (Table 1). The data showed that maximum increase in osmotic potential was observed in Sarsabz and Lu-26s followed by KTDH-22 and Bhittai. Although at this level of salinity, other three genotypes viz., V-7012, Khirman and Bakhtawar also exhibited increase in the osmotic potential over their respective controls, however this increase was much lower (less than 20%) as compared with the tolerant genotypes.

Effect of salinity on glycine-betaine and total sugars: Glycine-betaine is also one of the important osmo-protectants and its estimation serves as physiological marker for salt stress. Under saline conditions an overall increasing trend in glycine-betaine contents was noted in all genotypes (Table 2). Maximum increase was observed in Sarsabz and Bhittai showing obvious tolerance under salt stress. Minimum increase was observed in Bakhtawar followed by Khirman and V-7012.

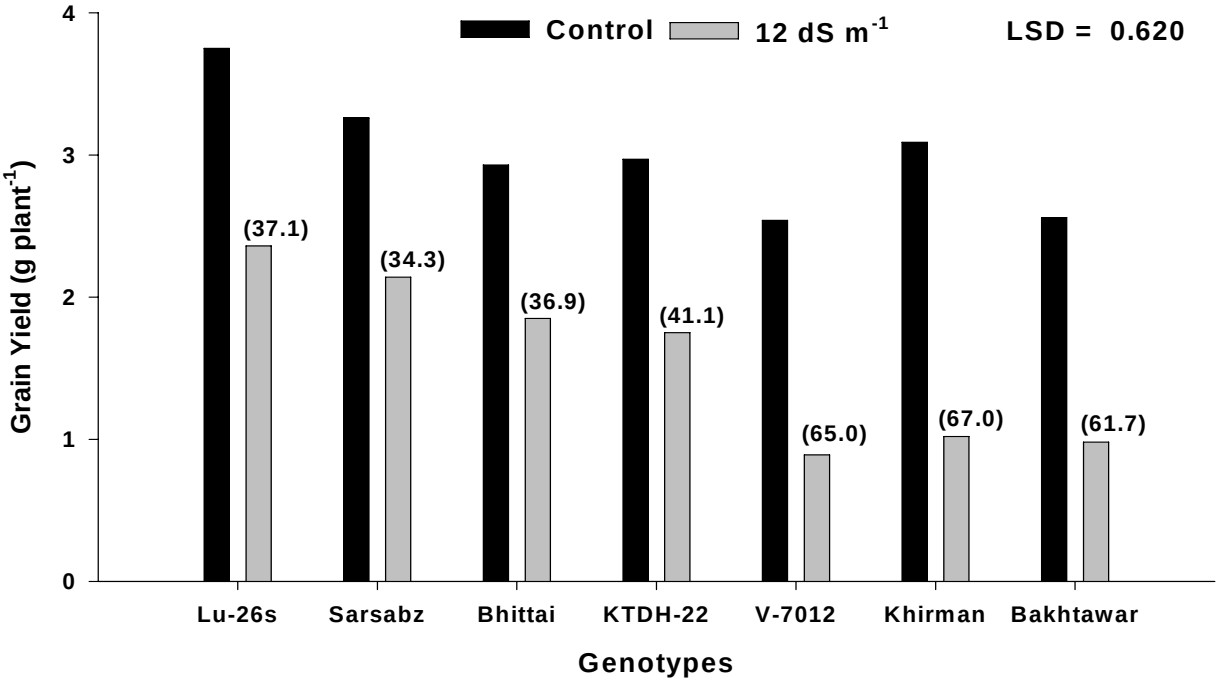

Fig. 1. Effect of salinity on the grain yield (g plant^{-1}) of different wheat genotypes. Data points represent means of three replicates (n=3). Values in the parenthesis indicate % reduction in the grain yield of treatment over control.

Table 1. Effect of salinity on the leaf area (cm^2) and osmotic potential (-MPa) of different wheat genotypes.

S. No.	Variety	Leaf area (cm^2)					Osmotic potential (-MPa)				
		Control		12 dS m^{-1}		% red.	Control		12 dS m^{-1}		% inc.
1.	Lu-26s	32.5	a	25.3	a	22.2	0.55	a	0.91	a	66.4
2.	Sarsabz	34.2	a	25.0	a	26.8	0.57	a	0.96	a	66.9
3.	Bhittai	32.3	a	24.0	a	25.8	0.62	a	0.93	a	49.2
4.	KTDH-22	31.0	ab	23.3	b	24.7	0.59	a	0.92	a	56.2
5.	V-7012	28.0	bc	17.5	b	37.5	0.59	a	0.70	b	18.0
6.	Khirman	27.3	c	18.3	b	32.9	0.60	a	0.70	b	17.5
7.	Bakhtawar	28.0	bc	16.0	b	42.9	0.58	a	0.68	b	17.2
Mean		30.5		21.3			0.59		0.83		
LSD (0.05)		3.36					0.053				

Table 2. Effect of salinity on glycine-betaine (μmol g^{-1} dry wt.) and total sugars (mg g^{-1} dry wt.) of different wheat genotypes.

S. No.	Variety	Glycine-betaine (μmol g^{-1} dry wt.)					Total sugars (mg g^{-1} dry wt.)				
		Control		12 dS m^{-1}		% inc.	Control		12 dS m^{-1}		% inc.
1.	Lu-26s	5.9	ab	26.0	c	341.9	8.3	a	22.3	a	168.9
2.	Sarsabz	5.4	b	28.7	a	435.7	8.1	a	22.0	a	172.8
3.	Bhittai	5.2	b	27.1	bc	417.0	8.1	a	20.9	ab	157.9
4.	KTDH-22	5.8	ab	28.4	ab	387.7	8.2	a	19.8	b	141.6
5.	V-7012	5.2	b	18.8	d	258.9	8.1	a	15.9	c	95.5
6.	Khirman	5.7	ab	18.1	d	214.9	8.2	a	15.9	c	94.1
7.	Bakhtawar	7.1	a	19.2	d	168.5	8.5	a	15.3	c	79.3
Mean		5.78		23.8			8.2		18.9		
LSD (0.05)		1.05					0.962				

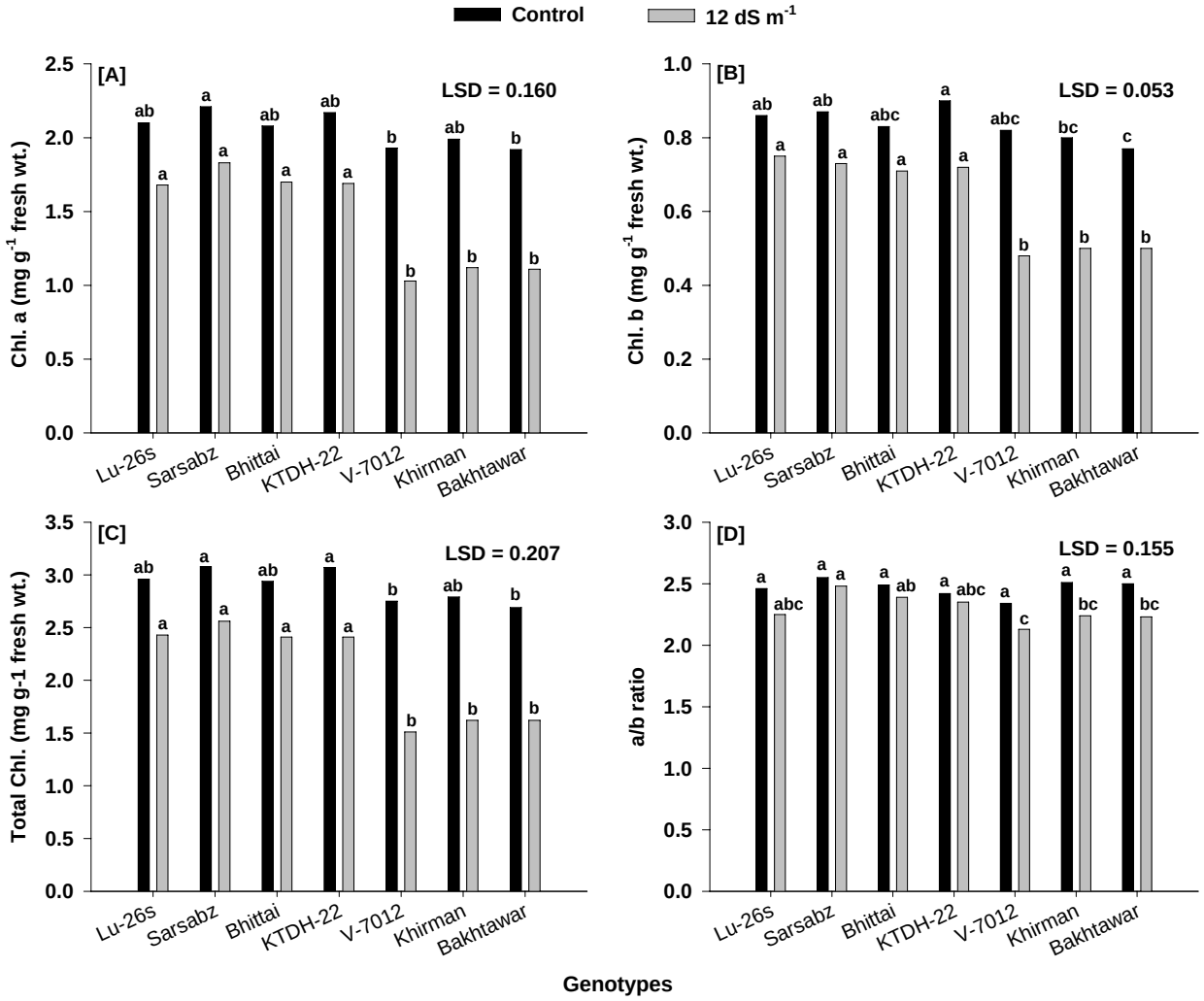

Fig. 2. Effect of salinity on the Chlorophyll a, b, total chlorophyll (mg g^{-1} fresh wt.) and a/b ratio of different wheat genotypes. Data points represent means of three replicates (n=3). Different letters indicate significant differences ($p \leq 0.05$) between treatment and control.

Under saline condition, increase in total soluble sugars ranged from 79.7 to 168.9 % with significant differences among genotypes (Table 2). After the imposition of salt stress more than 100% increase in sugar contents was noted in genotypes Lu-26s, Sarsabz, Bhittai and KTDH-22 when compared with their respective controls. However maximum increase was recorded in Sarsabz. The other three genotypes i.e. V-7012, Khirman and Bakhtawar exhibited less than 100% increase in total sugar with lowest sugar contents in genotype Bakhtawar.

Effect of salinity on chlorophyll contents: The data regarding variation in chlorophyll a contents are presented in Figure 2A which shows a significant variation among genotypes. In general, a declining trend was noted in all genotypes with the presence of excessive salts in the growth medium. Maximum reduction was noted in V-7012, Khirman and Bakhtawar however, genotypes Sarsabz, Bhittai and Lu-26s retained maximum chlorophyll a contents with less than 20% reduction. Application of salinity markedly reduced the chlorophyll b contents (Fig. 2B). All the genotypes showed less reduction in the contents of chlorophyll b than a under salt stress. Genotype Lu-26s showed better response and exhibited least reduction in chlorophyll b contents followed by Bhittai and Sarsabz.

The data regarding total chlorophyll contents in genotypes under investigation are presented in Fig. 2C. Imposition of salinity caused a marked decrease in total chlorophyll contents of all genotypes, however maximum reduction was noted in V-7012 followed by Khirman and Bakhtawar. Genotypes Sarsabz, Lu-26s and Bhittai, showed less than 20% reduction in total chlorophyll and proved to be tolerant to this salinity level. Any decrease or increase in chlorophyll a and b contents determines the change in their ratio. All the genotypes showed a slight declining trend in chlorophyll a/b ratio with increase in salinity (Fig. 2D), however maximum decrease was recorded in Khirman and Bakhtawar. Genotypes Sarsabz, KTDH-22 and Bhittai were tolerant to this salinity and exhibited negligible reduction in chlorophyll a/b ratio as compared with control.

Discussion

Plant responses to salinity are complex and depend upon a number of factors eg., duration of salinity, type of salts, developmental stage of plant at exposure, (Cramer, 2002; Saqib, 2002). In general, salinity seriously affects different growth parameters and yield of wheat like other field crops. Yield reduction may range from a slight loss to complete crop failure depending upon severity of the salinity problem (Chang & Sipio, 1991). In the present study a reduction in the grain yield of genotypes Khirman, V-7012 and Bakhtawar showed that these genotypes are sensitive to salinity while genotypes Sarsabz, Bhittai and Lu-26s gave better yield showing obvious salt tolerance. According to Kamkar *et al*., (2004) the salinity induced source limitation reduces yield primarily by a severe reduction in grain number and then by reduction in grain yield.

One immediate response of plant to elevated salinity is a decrease in the rate of leaf expansion, which results in a reduced leaf area. Results of the present study showed that a significant positive correlation exists between grain yield and leaf area under NaCl stress. It has been reported that common decrease in leaf expansion is associated with a loss in cell turgor rather than a salt-specific effect. However, Ball (1988) indicated that the common decrease in leaf expansion is not related to a loss in turgor pressure and is most likely a result of a change in hormonal signaling from roots to leaves. In the salt-sensitive genotypes, in which salt is not effectively excluded from the transpiration stream, salt will build up to toxic levels in the leaves, resulting in death of old leaves and injury to new leaves which may become succulent to dilute the salts (Munns & James, 2003).

Salt exclusion is the predominant mechanism of salt tolerance in non-halophytic plants where osmotic adjustment is achieved, either by taking up non-toxic inorganic solutes like K^+, Ca^{+2} and NO_3^- at an increased rate or by the synthesis of organic solutes (Serrano *et al*., 1999). An increase in osmotic potential is accompanied by more accumulation of organic and inorganic solutes, therefore the genotypes which have higher increase in osmotic potential can be regarded as more tolerant to salinity i.e., Lu-26s, Sarsabz, Bhittai and KTDH, whereas the genotypes which have less increase in osmotic potential i.e., V-7012, Khirman and Bakhtawar are susceptible to salinity. Water deficit in plants due to low external water potential is considered to be the first cause of growth inhibition under saline conditions (Munns & James, 2003). Salt tolerance and growth in a saline substrate; therefore require a decrease in intracellular water potential (Greenway & Munns, 1980) by increasing the quantity of osmotically active solutes in the tissue (Gorham *et al*., 1985).

Glycine-betaine, proline, D-sorbitol and D-pinitol are the common organic osmolites (Gorham *et al*., 1985). Glycine-betaine has been reported to accumulate in sunflower (Iqbal *et al*., 2008) and grasses (Akram *et al*., 2007) under water stress and saline

conditions, respectively. The results of present study clearly revealed that glycine-betaine accumulation was enhanced by salinity in all the wheat genotypes (Table 2). Studies by Meloni *et al*., (2004); Khan & Asim (1998) and Yeo (1998) reported the genotypic variation in glycine-betaine accumulation in *Prosopis alba* and cotton. Similarly Khan *et al*., (1995) also reported the accumulation of glycine-betaine under salinity in sorghum and recorded three fold more betaine contents in salt stressed plants of sorghum compared to that under non-saline environment. In the present study, glycine-betaine contents increased due to salinity and obvious genotypic variations were also observed. The genotypes Sarsabz, Bhittai and KTDH-22 had significantly higher values for glycine-betaine than the other genotypes. The results also showed that these genotypes had higher grain yields under salinity stress showing positive correlation between grain yield and glycine-betaine contents. Sairam *et al*., (2002) found that tolerant genotypes had higher glycine-betaine values under control and saline conditions compared to the susceptible ones, which had much lower contents of glycine-betaine.

Accumulation of total sugars/carbohydrates under salinity stress is a commonly noted phenomenon (Khan *et al*., 1995; Munns & James, 2003) and it has been reported to play an important role in osmotic adjustment under salinity stress in grasses (Akhtar *et al*., 2004). Results of the present study also indicated that with the application of salinity, accumulation of sugar was enhanced and maximum sugar was recorded in tolerant genotypes especially in Lu-26s and Sarsabz. Although sugar contents of the sensitive genotypes were also increased under saline conditions, however their level of accumulation was much lower compared to the tolerant genotypes. Similar results are also reported by Prado *et al*., (2000) and Vacher *et al*., (1994) who found an increase in the total sugar with progressive salinity increase in *Chenopodium quinoa* cotyledon and suggested that sugars could act as osmoregulators. Literature also indicated that the salt tolerant accessions of grasses had higher accumulation of sugars and maintained turgor by decreasing osmotic potential and showed better osmotic adjustment. In the present study accumulation of sugar was much higher in the tolerant genotypes, which created lower osmotic potentials in these genotypes and hence helped them to produce higher grain yields.

Salinity significantly reduces the total chlorophyll content depending on salt tolerance of plant species and tissue salt concentrations. According to Ashraf & McNeilly (1988), chlorophyll contents in salt-tolerant species increased, while in salt-sensitive species these were decreased. The reduction in chlorophyll contents is to be expected under stress; being membranous bound, its stability is dependent on membrane stability, which under saline condition seldom remains intact (Ashraf *et al*., 2005). The decrease in chlorophyll contents under saline conditions is reported by Iqbal *et al*., (2006); Ashraf *et al*., (2005). Our results are in agreement with these scientists; where chlorophyll contents in all genotypes decreased. There are, however some reports where an increase in chlorophyll contents was observed in rice genotypes (Alamgir & Ali, 1999). Another interesting phenomenon is the shift in chlorophyll a/b ratio (Fig. 2D). It has been reported by many workers that under saline conditions, chlorophyll b decreases more than chlorophyll a, thus shifting the ratio in favour of chlorophyll a. In the present study the chlorophyll a/b ratio varied among the genotypes with overall more increase in a/b ratio of tolerant genotypes as compared with the susceptible ones. Ashraf & Mehmood (1990) on the other hand, found a decrease in chlorophyll a/b ratio in three out of four brassica species.

It is concluded that on the basis of yield reduction, four genotypes viz., Lu-26s, Sarsabz, Bhittai and KTDH-22 were found to be salt tolerant whereas genotypes V-7012, Khirman and Bakhtawar could be designated as sensitive ones. The tolerant genotypes also maintained higher leaf area, osmotic potential, glycine-betaine, total sugar and chlorophyll contents under saline conditions.

Acknowledgements

The authors are highly thankful to the wheat breeders of Plant Breeding and Genetics Division, NIA, Tandojam for supplying the seeds of wheat genotypes and Mr. Saqib Ali Memon for help in composing the manuscript.

References

Akhtar, J., K. Mahmood, K.A. Malik, A. Mardana, M. Ahmad and M.M. Iqbal. 2004. Effects of hydrogel amendment on water storage of sandy loam and loam soils and seedling growth of barley, wheat and chickpea. *Plant Soil Environ.*, 50: 463-469.

Akram, M., M.A. Malik, M.Y. Ashraf, M.F. Saleem and M. Hussain. 2007. Competitive seedling growth and K^+/Na^+ ratio in different maize (*Zea mays* L.) hybrids under salinity stress. *Pak. J. Bot*., 39(7): 2553-2563.

Alamgir, A.N.M. and M.Y. Ali. 1999. Effect of salinity on leaf pigments, sugar and protein concentrations and chloroplast ATPase activity of rice (*Oryza sativa* L.). *Bangladesh J. Bot*., 28(2): 145-149.

Anonymous. 2009. *Agricultural Statistics of Pakistan* 2007-2008. Government of Pakistan, Ministry of Food and Agriculture (Economics Wing), Islamabad, p. 3.

Anonymous. 2009. Annual meeting of API Standing Committee on wheat crop. Agricultural Policy Institute, Ministry of Food and Agriculture, Govt. of Pakistan, 14 July, Islamabad.

Ashraf, M. and S. Mehmood. 1990. Response of four brassica species to drought stress. *Env. Exp. Bot*., 30: 93-100.

Ashraf, M. and T. McNeilly. 1988. Variability in salt tolerance of nine spring wheat cultivars. *Crop Sci*., 160: 14-21.

Ashraf, M.Y., M. Ashraf and G. Sarwar. 2005. Physiological approaches for improving plant salt tolerance. In: *Crops growth, quality and biotechnology* (Ed.): R. Dris. WFL Publisher, Helsinki, pp. 1206-1227.

Ball, M.C. 1988. Salinity tolerance in the mangroves, *Aegceras corniculatum* and *Avicennia marina* L. Water use in relation to growth, carbon partitioning and salt tolerance. *Aust. J. Plant Physiol*., 15: 447-464.

Chang, M.H. and Q.A. Sipio. 1991. Salt tolerance of two varieties. *J. Drain. Recl*., 3: 36-39.

Cramer, G.R. 2002. Sodium-calcium interactions under salinity stress. In: *Salinity: Environment – Plants – Molecules* (Eds.): A. Läuchli and U. Lüttge, Kluwer Academic Publishers, Netherlands. pp. 327-339.

Ghassemi, F., A.J. Jakeman and H.A. Nix. 1995. Salinization of land and water resources. Centre for resource and environmental studies. *The Australian National University, Canberra Act. 0200*, pp, 526.

Gorham, J., R.G. Wyn Jones and E. McDonnell. 1985. Some mechanism of salt tolerance in crop plants. *Plant Soil*., 89(1-3): 15-40.

Greenway, H. and R. Munns. 1980. Mechanism of salt tolerance in non-halophytes, *Ann. Rev. Plant Physiol*., 31: 149-190.

Grieve, C.M. and S.R. Gratan. 1983. Rapid assay for determination of water soluble Quaternary ammonium compounds. *Plant and Soil*., 70: 303-307.

Iqbal, N., M. Ashraf and M.Y. Ashraf. 2008. Glycine-betaine an osmolyte of interest to improve water stress tolerance in sunflower: water relation and yield. *South African J. Bot*., 74: 274-281.

Iqbal, N., M.Y. Ashraf, Farrukh Javed, Vicente Martinez and Kafeel Ahmad. 2006. Nitrate reduction and nutrient accumulation in wheat (*Triticum aestivum* L.) grown in soil salinization with four different salts. *J. Plant Nutr*., 29: 409-421.

Kamkar, B., M. Kafi and A. Nassiri Mahallati. 2004. Determination of the most sensitive developmental period of wheat (*Triticum aestivum*) to salt stress to optimize saline water utilization. 4th Int. *Crop Sci. Cong*., 6 p.

Khan, A.H., M.Y. Ashraf and A.R. Azmi 1992.Osmotic adjustment in wheat. A response to water stress. *Pak. J. Sci. Ind. Res*., 36: 151-155.

Khan, M.A. 2009. Salt tolerance in wheat. Ph.D. Thesis submitted to University of Sindh, Jamshoro..

Khan, M.G., M. Silberbush and S.H. Lips. 1995. Physiological studies on salinity and nitrogen interaction in Alfa alfa. *J. Plant Nutr*., 17: 93-99.

Khan, M.I. and F. Asim. 1998. Salinity tolerance of wheat through seed treatment with diluted and potentized sodium chloride. *Pak. J. Bot*., 30: 145-149.

Lichtenthaler, H.K. 1987. Chlorophyll and carotenoids pigments of photosynthetic biomembranes. *Methods Enzymol*., 148: 350-382.

Maas, E.V. 1986. Salt tolerance of plants. *Appl. Agri. Res*., 1: 12-26.

Meloni, D.A., M.R. Gulotta, C.A. Martinez and M.A. Oliva. 2004. The effects of salt stress on growth, nitrate reduction and proline and glycine-betaine accumulation in *Prosopis alba*. *Braz. J. Plant Physiol*., 16(1): 39-46.

Munns, R. and R.A. James. 2003. Screening methods for salinity tolerance: A case study with tetraploid wheat. *Plant Soil*., 253: 201-218.

Prado, F.E., C. Boero, M. Gallardo and J.A. Gonzalez. 2000. Effect of NaCl on germination, growth and soluble sugar. *Bot. Bull Acad. Sin*., 41: 27-34.

Qureshi, R.H. 1993. Alternative strategies for tackling the soil salinity problem. Dept. Soil Sci., Univ. Agri., Faisalabad, Pakistan, p. 545-549.

Rafiq, M. 1990. Soil resources and soil related problems in Pakistan. In: *Soil physics application under stress environments*, (Eds.): M. Ahmad, M.E. Akhtar and M.I. Nizami. BARD, Islamabad, Pakistan. pp. 16-23.

Riazi, A., K. Matsuda and A. Arsalan. 1985. Water stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. *J. Exp. Bot*., 36: 1716-1725.

Sairam, R.K., K.V. Rao and G.C. Srivastava. 2002. *Plant Sci.*, 163(5): 1037-1046.

Saqib, M. 2002. Selection and characterization of wheat genotypes against salinity and water logging. Ph.D. Thesis. Department of Soil Science University of Agriculture, Faisalabad.

Serrano, R., J.M. Mulet, G. Rios, J.A. De Marquez, I. Larrinoa, M.P. Leube, I. Mendizabal, A.P. Ahuir, M. Proft, R. Ros and C. Montesinos. 1999. A glimpse of the mechanisms of ion homeostasis during salt stress. *J. Exp. Bot*., 50: 1023-1036.

Vacher, J.J., J. Dizes, G. Spindola and C. del Castillo. 1994. VIII Congreso International de Sistemas Agropecuarios Andinos. Valdivia-Chile., 22: 20.

Wild, A. 2003. *Soils, Land and Food: Managing the land during the 21st century - An Outline*. Cambridge, UK Cambridge University Press, p. 8-9.

Wyn Jones R.G. and R. Storey. 1981. *Betaines. In: Physiology and Biochemistry of Drought Resistance in Plants*. (Eds.): L.G. Paleg and D. Aspinall. (Eds.). Academic Press, Sydney. pp. 171-204.

Yeo, A. 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Exp. Bot*., 49: 915-929.

(Received for publication 3 September 2009)